©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

$\dot{V}_{max}$ As a Measure of h_∞. A Contribution to Membrane Kinetics

$\dot{V}_{max}$ als Maß für h_∞. Ein Beitrag zur Membrankinetik

By A. KOLLER and H. MOSER

Institut für Zoologie, Abteilung Zoophysiologie, Technikerstraße 25, A-6020 Innsbruck, Austria

With 4 Figures

(Vorgelegt in der Sitzung der mathem.-naturw. Klasse am 17. Jänner 1986 durch das k. M. W. WIESER)

Zusammenfassung

Die Methode der Spannungsklemme-Technik scheint bei bestimmten Präparaten, wie beispielsweise dem Herzmuskel, nicht geeignet, um Auskunft über den Natrium-Membranstrom (I_{Na}) und einige seiner zugrunde liegenden Komponenten zu geben, wie z. B. die „steady state Na-Inaktivierung" (h_∞). Möglicherweise erhält man aber dieses Wissen durch Auswertungen von $\dot{V}_{max}$, der maximalen Anstiegsgeschwindigkeit eines Aktionspotentials. In der Literatur sind diesbezüglich unterschiedliche Meinungen vertreten, die ihre Argumente überwiegend aus Computer-Simulationsexperimenten beziehen. Dabei wurden mit unterschiedlichen Modellen, einerseits dem modifizierten Aktionspotential des Riesenaxons vom Tintenfisch, andererseits an einem bestimmten Herzmodell, Berechnungen durchgeführt. Aus unseren Simulationen schließen wir, daß die Kontroverse um die Anwendbarkeit der $\dot{V}_{max}$-Messungen sich sowohl auf die unterschiedlichen Arten der angewendeten Reizung als auch auf die Variablen der Membrankinetik zurückführen lassen.

Abstract

In a variety of preparations, especially in the myocardium, I_{Na} and some of its component properties, such as the steady state sodium inactivation (h_∞), cannot be obtained by measurements with the voltage clamp method but possibly by evaluation of $\dot{V}_{max}$, the maximum upstroke velocity of an action potential. There exist opposing views in this matter in the literature which are mainly based on computer simulations done on the modified squid axon membrane action potential and a specified cardiac model. From our simulations we conclude that the controversy about the applicability of $\dot{V}_{max}$ measurements are due to different modes of stimulation as well as to the kinetic variables of the different models used.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

Introduction

Although recent voltage clamp measurements in cardiac preparations are in accordance with earlier investigations (SCHOENBERG and FOZZARD, 1979; HAAS and BROMMUNDT, 1980) there remains some doubt about the correctness of estimates of the sodium current (I_{Na}) in the sheep Purkinje strand (for literature see: LEVIS et al., 1983), atrial or ventricular myocardium and in certain other preparations (e.g. skeletal muscle). The doubts are mainly due to the structural complexities of the preparations because of intracellular clefts. Presently it seems as if reliable I_{Na} estimates cannot be obtained with the voltage clamp method in these preparations, even if the current is substantially reduced by experimental manipulations (LEVIS et al., 1983).

An alternative approach in obtaining information on I_{Na} and some of its component properties was introduced by WEIDMANN (1955 a,b), who evaluated measurements of $\dot{V}_{max}$, the maximum upstoke velocity of an action potential. Since then this method was used in a variety of preparations, e.g. in the myocardium (BAER et al., 1976; CHEN et al., 1975; GETTES and REUTER, 1974). Controversial views have been discussed whether the measurements of $\dot{V}_{max}$ suffice to obtain information on I_{Na} and its component properties such as the maximum sodium conductance ($\overline{G}_{Na}$) and on the steady state sodium inactivation (h_∞). From their constant current stimulations on the modified squid axon membrane action potential model STRICHARTZ and COHEN (1978) concluded that h_∞ cannot be derived from $\dot{V}_{max}$ and question the general applicability of cardiac membran models. This point of view was opposed by WALTON and FOZZARD (1979) who showed that in the cardiac membrane model of Mc. ALLISTER et al. (1975) constant latency between the stimulus and the time of $\dot{V}_{max}$ is required in order to derive h_∞ from $\dot{V}_{max}$.

In our simulation studies we try to clarify whether the opposing views are due to different kinds of stimulations of the models and/or due to the different models used. Parts of the results have been published in abstract form (KOLLER, 1981; KOLLER and MOSER, 1981).

Methods

Computer simulations of $\dot{V}_{max}$ values were done on the model of the uniform squid axon membrane action potential based on equations of HODGKIN and HUXLEY (1952). The equations were modified by keeping potassium and leak currents to zero as proposed by STRICHARTZ and COHEN (1978). The programs were written in FORTRAN and run on a Control Data Corp. computer at the Rechenzentrum in Innsbruck. According to WALTON and FOZZARD (1979) a Runge-Kutta numerical integration method was applied (RALSTON and WILF, 1960) with a variable time increment adjusted to give a constant degree of accuracy (10^{-5} mV) while decreasing the time of evaluation per stimulation.

The model had a resting potential of − 60 mV Conditioning de- or hyperpolarizing prepulses with a duration of 200 ms were given before a

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

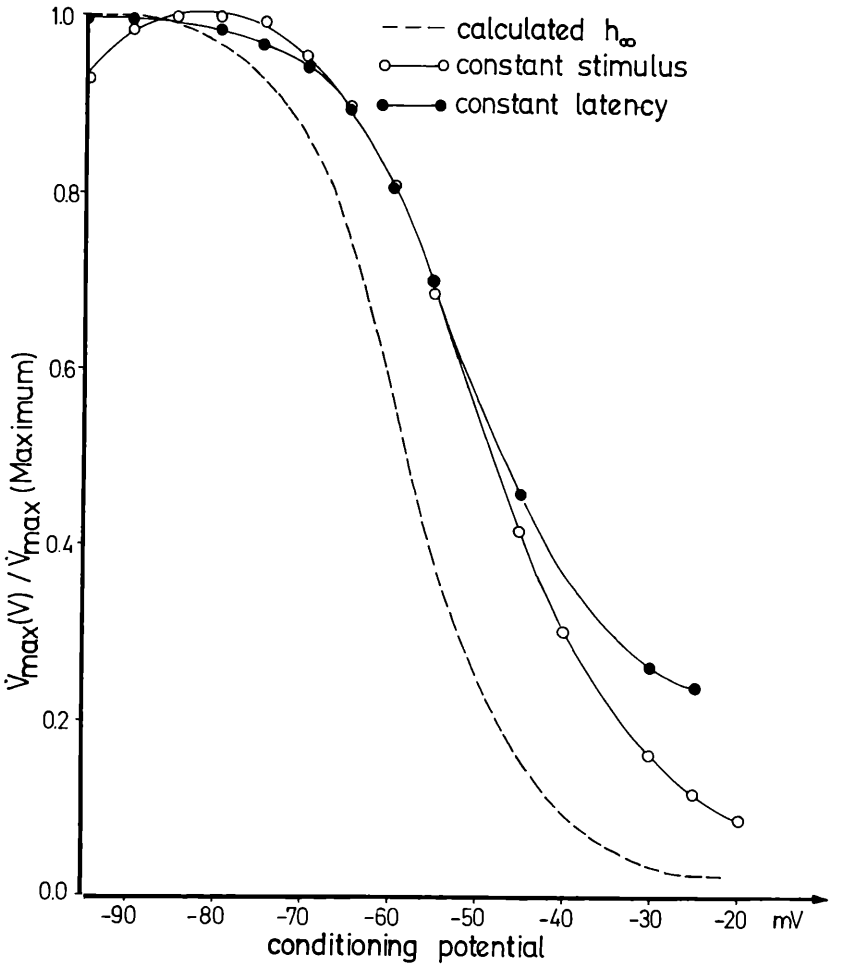


Fig. 1: Graph of fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration from resting potential relative to the largest $\dot{V}_{max}$ value (− 95 mV for constant current intensity stimulus curve, − 85 mV for constant latency curve). The intensity of the stimulus was either 8 mA/cm² after all conditioning potentials or it was varied to obtain constant latency (2 ms). Stimulus duration was 4 μs in all trials. The dashed control curve of steady state h values was calculated from the equations in the model.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

stimulus was set to trigger the action potential. The stimulus duration was 4 μs. Stimulus intensity was either set to 8 mA/cm² or it was adjusted so as to initiate action potentials with a constant latency of 2 ms between stimulus and the time of $\dot{V}_{max}$. A temperature of 6°C was assumed for the modified squid axon model unless otherwise specified.

The plots represent h_∞ curves and demonstrate the relationship between fractional $\dot{V}_{max}$ values which were obtained after conditioning voltage steps as indicated. The dashed control curve corresponds to the steady state $h(h_\infty)$ value calculated from the explicit equations in the model.

Results

Fig. 1 illustrates h_∞ curves obtained during stimulation with constant current intensity (8 mA/cm²) or constant latency (2 ms) in comparison to the control. Neither of the two modes of stimulation gave an acceptable fit to the control response. At conditioning potentials more negative than about − 80 mV, stimulation with constant current intensity always resulted in a decrease of the fractional $\dot{V}_{max}$ value while in the case of constant latency stimulation the curve approached to that of the control (compare with Fig. 2 from WALTON and FOZZARD, 1979). In further investigations (not shown) the stimulus duration was prolonged to 1 ms instead of 4 μs at constant intensity. The results showed negligible deviations from the main part of the 4 μs curve.

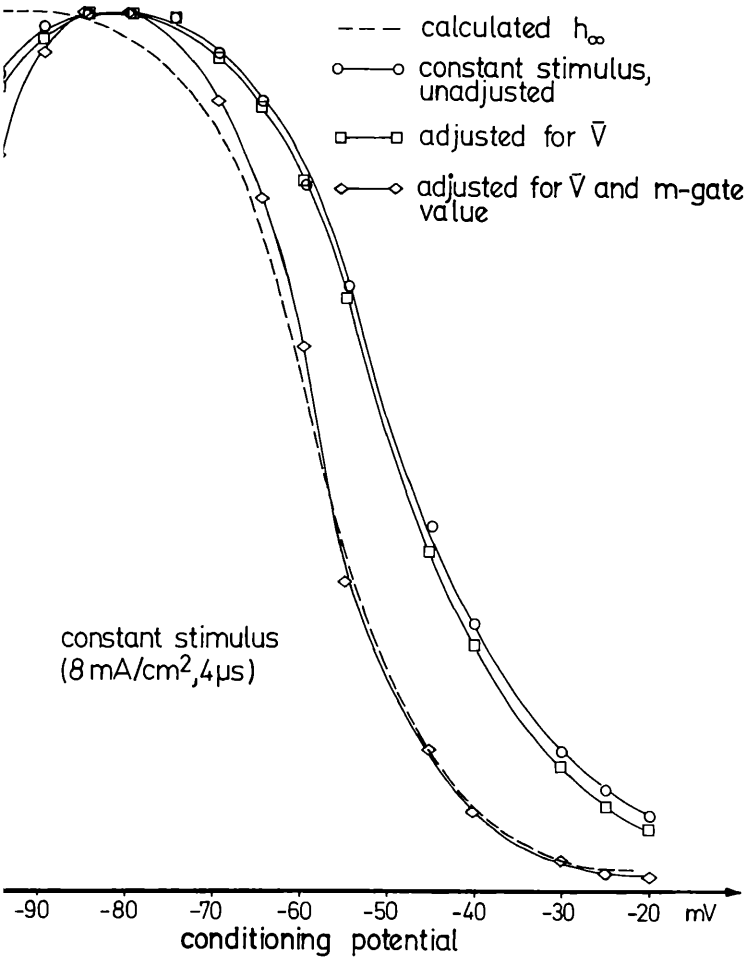
At the potential and the moment at which $\dot{V}_{max}$ occurs variations of several parameters are to be expected. To account for this corrections were performed according to WALTON and FOZZARD (1979) for $\overline{V}$, the membrane potential at which $\dot{V}_{max}$ occurred, and the m-gate value (sodium activation variable) at $\dot{V}_{max}$. In this respect is should be mentioned that these corrections can be done in simulations only. In a biological preparation neither latency nor $\overline{V}$ and m^3 can be manipulated separately (HAUSWIRTH, personal communication, 1984).

Effects of such corrections in simulations executed with constant stimulus intensity are shown in Fig. 2. While there was little difference between the unadjusted curve and that corrected for $\overline{V}$, a pronounced change was found for the additional corrections of the m-gate value. Nevertheless, the fit to the control was still not satisfactory, especially when the hyperpolarizing range of the conditioning potential was considered.

The same kind of corrections was performed during simulations with constant latency. As shown in Fig. 3 the corrections for $\overline{V}$ and the m-gate value resulted in a close approximation to the control curve (compare with Fig. 3 from WALTON and FOZZARD, 1979).

Both modes of stimulation revealed evidence that the fractional $\dot{V}_{max}$ curve was highly sensitive to the m-gate value which indicated that the activation kinetics of the sodium channel is one critical parameter to account for the validity of $\dot{V}_{max}$ measurements. To demonstrate its importance simulations were performed at different temperatures, keeping all other parameters constant. The results are shown in Fig. 4.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at



raph of fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration from otential relative to the value at −95 mV conditioning step. Stimulus intensity was ^{2} and lasted for 4 μs. Corrections for $\bar{V}$ and for both $\bar{V}$ and m-gate values are also shown.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

In comparison to simulations performed at 6°C the ratio of τ_h and τ_m (the time constant [τ] of the sodium inactivation [h] and activation [m]) is increased at 25°C which resulted in a shift to the control curve.

Discussion

In contrast to the cardiac membrane model (Mc. ALLISTER et al., 1975; WALTON and FOZZARD, 1978) in which constant latency stimulation alone suffice for a good fit between control and test h_∞ curve, there seem to be two parameters essential for the same effect in the modified squid axon model (HODGKIN and HUXLEY, 1952; STRICHARTZ and COHEN, 1978): (1) the mode of stimulation as well as (2) the kinetics of the m-gate value.

At constant current stimulation there was only a rather limited range in which the test curves fitted to the control. Especially at conditioning potentials more negative than about − 70 mV the deviations got more and more apparent. Constant latency stimulation, however, revealed the basis for a good fit over the hole range of conditioning potentials performed, which was between about −20 and −95 mV.

Under the conditions of constant latency stimulation a correction for the sodium activation value (m^3) at the moment of $\dot{V}_{max}$ had to be introduced in the modified squid axon model to improve the fit to the control curve sufficiently. It should be noted that V_{max} is much more sensitive to variations of m than either $\overline{V}$ or h (the role of τ_h is extensively discussed by WALTON and FOZZARD, 1979) because of its cubic rather than linear dependency (cf. COHEN et al., 1981). Such a correction does not seem to be necessary for certain cardiac membrane models which might be caused by the *ad hoc* assumptions concerning the sodium channel kinetics. Our simulations on the modified squid axon model indicated that an increased ratio of the inactivation and activation constants (τ_h/τ_m) *per se* produced results more consistent with those of WALTON and FOZZARD (1979) for their cardiac model simulations. If it is assumed for analytical procedures that $\tau_m = 0$ and $\tau_h = \infty$ (cf. COHEN et al., 1981) the kinetic conditions tend automatically to those which are closer to the cardiac than to the modified squid axon model. The ratios τ_h/τ_m so far given are 3−5 for the squid axon and 28−104 for the cardiac membrane in the range of − 40 to 0 mV (Mc. ALLISTER et al., 1975).

The main drawback of constant current stimulation seems the loss of the control over the kinetics of the sodium channel. Under biological conditions there is neither $\tau_m = 0$ nor $\tau_h = \infty$, but constant latency stimulation offers the opportunity to control τ.

Our conclusions are: the opposing views about the applicability of $\dot{V}_{max}$ measurements for determination of I_{Na}, $\overline{G}_{Na}$ or h_∞ are due to different modes of stimulation as well as to the kinetic variables of different models of simulation. Thus results should be assessed with caution when $\dot{V}_{max}$ measurements were used for determination of these parameters.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

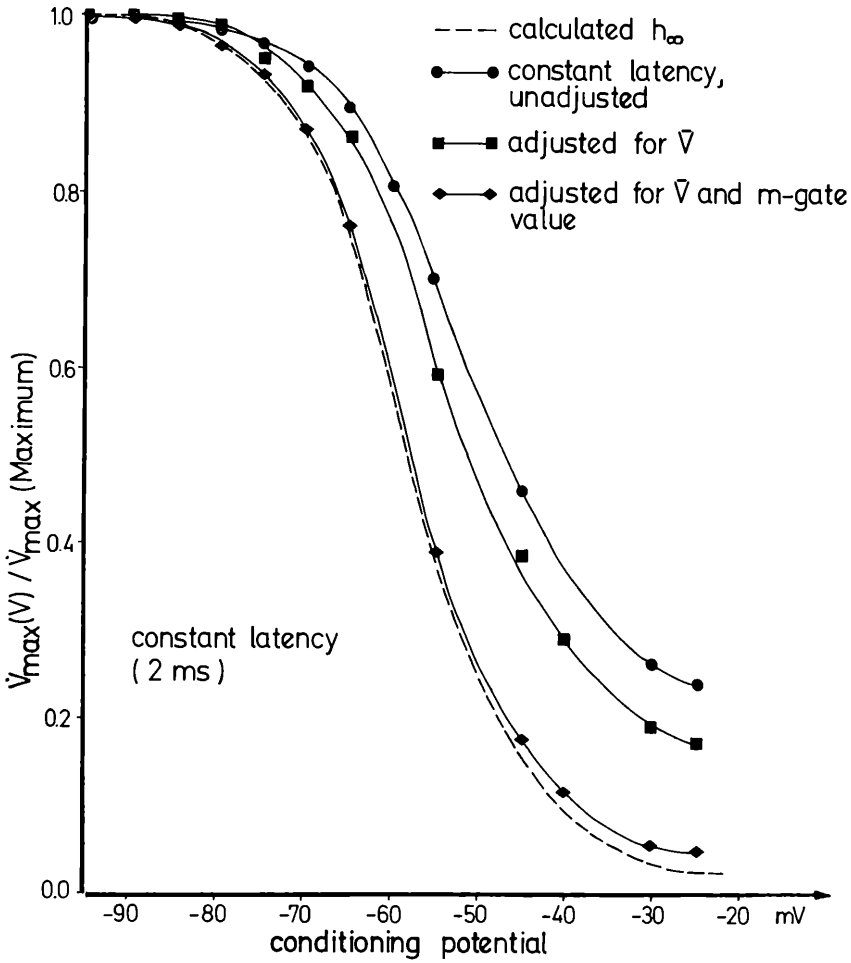


Fig. 3: Graph of fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration from resting potential relative to the value at − 85 mV conditioning step. Stimulus intensity varied in order to obtain constant latency at all potentials. Correction for $\bar{V}$ and for both $\bar{V}$ and m-gate values are included, too.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

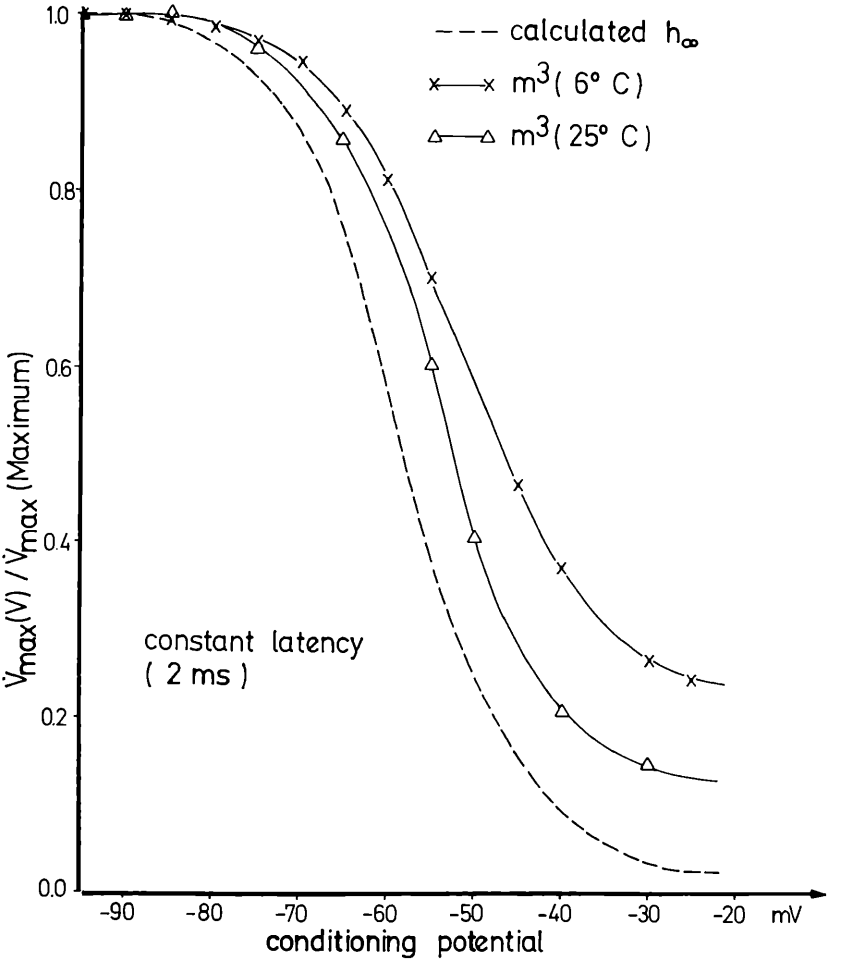


Fig. 4: Graph of fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration from resting potential relative to the value at − 85 mV conditioning step. The effect of temperature on m^3 is shown.

©Akademie d. Wissenschaften Wien; download unter www.biologiezentrum.at

Acknowledgements

We wish to thank Profs. O. HAUSWIRTH (Bonn), J. HOYER (Wien) and M. WALTON (Chicago) for their comments on an early version of this paper. Thanks to Prof. W. WIESER (Innsbruck) for encouragement during this work and to members of the Rechenzentrum for their help. This work was partially supported by the „Fonds zur Förderung der wissenschaftlichen Forschung in Österreich", project 3315.

References

BAER, M., BEST, P. M. & REUTER, H. (1976): Voltage-dependent action of tetrodotoxin in mammalian cardiac muscle. Nature *253*, 344–345.

CHEN, C. M., GETTES, L. S. & KATZUNG, B. G. (1975): Effect of lidocaine and quinidine on steady state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. Circ. Res. *37*, 20–29.

COHEN, I., ATTWELL, D. & STRICHARTZ, G. (1981): The dependence of the maximum rate of rise of the action potential upstroke on membrane properties. Proc. Roy Soc. Lond. *B 214*, 85–98.

GETTES, L. S. & REUTER, H. (1974): Slow recovery from inactivation of inward currents in mammalian myocardial fibres. J. Physiol. *240*, 703–724.

HAAS, H. G. & BROMMUNDT, G. (1980): Influence of intercellular clefts on potential and current distribution in a multifiber preparation. Biophys. J. *30*, 327–350.

HODGKIN, A. L. & HUXLEY, A. F. (1952): A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. *177*, 500–544.

HONDEGHEM, L. M. (1978): Validity of $\dot{V}_{max}$ as a measure of the sodium current in cardiac and nervous tissues. Biophys. J. *3*, 147–152.

KOLLER, A. (1981): $\dot{V}_{max}$ as measure of h depends on τ_h/τ_m. Pflüg. Arch. *391*, R 13.

KOLLER, A. & MOSER H. (1981): $\dot{V}_{max}$ as a measure of h_∞. Pflüg. Arch. *389*, R 11.

LEVIS, R. A., MATTHIAS, R. T. & EISENBERG, R. S. (1983): Electrical properties of sheep Purkinje strands. Electrical and chemical potentials in the clefts. Biophys. J. *44*, 225–248.

Mc. ALLISTER, R. E., NOBLE, D. & TSIEN, R. W. (1975): Reconstruction of the electrical activity of cardiac Purkinje fibres. J. Physiol. *251*, 1–59.

RALSTON, A. & WILF, H. A. (1960): Mathematical Methods for Digital Computers. Wiley. New. York.

SCHOENBERG, M. & FOZZARD, H. A. (1979): The influence of intercellular clefts on electrical properties of sheep cardiac Purkinje fibers. Biophys. J. *25*, 217–234.

STRICHARTZ, G. R. & COHEN, I. (1978): $\dot{V}_{max}$ as a measure of $\overline{G}_{Na}$ in nerve and cardiac membranes. Biophys. J. *23*, 153–156.

WALTON, M. & FOZZARD, H. A. (1979): The relation of $\dot{V}_{max}$ to I_{Na}, $\overline{G}_{Na}$ and h_∞ in a model of the cardiac Purkinje fiber. Biophys. J. *25*, 407–420.

WEIDMANN, S. (1955a): The effect of cardiac membrane potential on the rapid availability of the sodium carrying system. J. Physiol. *127*, 213–224.

WEIDMANN, S. (1955 b): Effects of calcium ions and local anaesthetics on electrical properties of Purkinje fibres. J. Physiol. *129*, 568–582.